

The Rh Factor in Rural Practice

Responsibility of the General Practitioner

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SUMMARY

As the incidence of erythroblastosis fetalis is as high in rural as in urban areas, it behooves physicians in rural areas to anticipate the disease, to make special antepartum studies to determine the Rh status of obstetrical patients, and to be prepared to treat the affected baby if the aid of a specialist and special facilities are not obtainable.

Exchange transfusions have been carried out, by means described, in a small rural hospital.

ERYTHROBLASTOSIS fetalis, or hemolytic disease of the newborn due to Rh incompatibility between mother and fetus, is not limited to large medical centers. The emergency confronts physicians in rural areas.*

The incidence of erythroblastosis is usually given as one in 200 pregnancies,⁸ and an affected baby has less than a 50 per cent chance to survive without treatment.

The purpose of this presentation is to review fundamentals which have practical clinical application to the management of Rh problems in rural practice, and to outline a plan for management which has been successfully used in a rural area.

GENETICS AND IMMUNOLOGY

All human blood may be divided into four main groups according to the presence, or absence, in the erythrocyte of one or both of the A and B agglutinogens. These are not the only antigenic substances which may be present in the erythrocytes in human blood, and among the others is the antigen called the Rh factor.

The Rh factor is inherited. It is a Mendelian dominant characteristic. The fetus will be Rh-positive if either parent contributes the dominant Rh-positive gene. If the same factor is in both chromosomes the person is homozygous; if otherwise, heterozygous. If a homozygous Rh-positive male marries an Rh-negative female, all the children will be Rh-positive.

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* In California in 1949 there were 157 recorded infant deaths attributed to erythroblastosis and only nine to syphilis. In 1949 the incidence of infant deaths due to erythroblastosis was the same in rural areas as in urban areas of the state.

If he is heterozygous, half of the children will be Rh-positive and half of them Rh-negative.

In 85 per cent of persons (95 per cent of Negroes) the erythrocytes contain the Rh factor. These persons are Rh-positive. Conversely, 15 per cent do not have the factor and are called Rh-negative. It follows that exposure of an Rh-negative person to the antigenic Rh factor in erythrocytes of Rh-positive persons might stimulate production of Rh-antibody substances in the Rh-negative person. If this occurs, the Rh-negative person is said to be sensitized or iso-immunized.

There are two ways by which an Rh-negative female becomes sensitized: By receiving injections of Rh-positive blood, and by repeated pregnancies bearing an Rh-positive fetus. Pregnancy is a less potent immunizing factor than is injection of Rh-positive blood. There is in general a 1:30 chance that an Rh-negative woman will become sensitized.⁷ There is only a 1:1,000 chance for immunization by pregnancy alone in the primipara but for the second and third pregnancies the chances are 1:40, and they increase to 1:12 for the fifth and sixth pregnancies. In contrast, antibodies develop in about 50 per cent of Rh-negative women with a history of blood transfusion. If this happens before the first pregnancy, it is likely the first child will have severe erythroblastosis.

ESSENTIAL LABORATORY TESTS

When there are no clinical laboratory services available, tests for the Rh factor and erythroblastosis can be reduced to two: Typing for the Rh factor, and the Coombs test. Rh typing identifies the Rh-negative mothers, and the Coombs test on cord blood of the infant is a diagnostic test for erythroblastosis. These inexpensive procedures physicians can do personally, preferably after observing them done in experienced hands.

While tests for Rh antibodies in the maternal circulation are desirable when laboratory help is available, they are not suitable as an office procedure.

Typing for the Rh Factor: One drop of commercial[†] anti-Rh serum is added to two drops of fresh blood being tested. These are mixed on the warm glass plate of an Rh view box and the plate tilted back and forth slowly. If clumping occurs, it is complete in one to three minutes. Agglutination means the blood is Rh-positive, no agglutination that it is Rh-negative.

[†] Materials used are available from American Hospital Supply, Lederle, Ortho Pharmaceutical, and others.

The Coombs Test: Two drops of Coombs' anti-human-globulin reagent (Ortho) is added to two drops of a 2 per cent saline solution suspension of washed erythrocytes being tested. These are mixed gently in a small test tube. If the cells are heavily coated with blocking antibodies, agglutination will occur almost immediately. If weakly coated, incubation at room temperature for ten minutes followed by slow centrifugation will cause agglutination. A positive result in the Coombs test means erythroblastosis; a negative result may not rule it out.

Although the significance of Rh antibody determination will be discussed, the techniques will not be described since they require a well equipped laboratory. In general, laboratory determinations made before delivery cannot be accurately correlated with the certainty of occurrence of erythroblastosis in the fetus or with the severity of the condition if it does develop.⁶ Mothers of most severely affected infants usually have high antibody titres, yet many in whom sensitization is demonstrated deliver an unaffected baby.

There are two types of Rh antibodies. One is the agglutinin, or complete antibody, which in its specific reaction with the Rh-positive erythrocytes causes complete hemolysis of the cell. It is sometimes designated the "saline fraction," as it is demonstrated in a saline medium. The other, the blocking antibody, or albumin fraction, is sometimes called the incomplete antibody since it attaches to, or coats, the surface of the fetal erythrocyte without causing hemolysis. It can be activated to complete the hemolytic reaction by addition of an X-protein factor (conglutinin) found in human plasma or bovine albumin. The blocking antibody is thought by some investigators to protect the fetal erythrocyte it coats, since it appears to block direct hemolytic action of the circulating agglutinin.

MANAGEMENT OF OBSTETRICAL RH PROBLEMS IN RURAL AREAS

The suggested outline for management of obstetrical Rh problems in rural areas is based upon three possible situations depending on the presence or absence of laboratory and specialty help:

1. Management when neither laboratory nor specialty help is available:

(a) On the first visit a careful, complete obstetrical and transfusion history should be obtained.

(b) Rh typing of each obstetrical patient should be carried out. (Fifteen per cent will be Rh-negative.)

(c) The Coombs test should be applied to every infant born to an Rh-negative mother.

(d) If the Coombs test result is positive and criteria for a diagnosis of erythroblastosis are present, a blood transfusion for the infant must be considered. If possible, arrangements should be made for an interested pediatrician to treat the baby. Expense and time involved in bringing a specialist to the baby are often prohibitive. The same is true of transfer of the baby to a medical center, plus the

fact that travel is hazardous for the baby. Another alternative would be to send the mother to a center to await delivery. This is usually impossible. Faced with these problems, the physician is likely to be left with a choice of attempting the transfusion or standing helplessly by with the infant's life at stake.

2. Management when laboratory but no specialty services are available:

(a) A careful obstetrical and transfusion history.

(b) All obstetrical patients should be referred to the laboratory for Rh typing and routine determinations of blood factors. Mailing containers may be used for sending specimens of blood to the laboratory.

(c) If the blood is Rh-negative, antibody determinations should be carried out. The presence of antibodies early in pregnancy usually indicates sensitization with carry-over from previous exposure to the Rh antigen.

(d) Titration tests should be repeated at the seventh month. If results are negative, no more tests need be done. If positive, titrations should be determined again at the eighth month. A rise in titre may be significant.

(e) The cord blood of infants delivered of Rh-negative mothers should be subjected to the Coombs test.

(f) Arrangements should be made for transfusion if it appears the procedure will be necessary.

3. Management when laboratory and specialty help are both available:

(a) Preliminary tests as previously outlined.

(b) Consultation with appropriate specialists to form a team prepared to carry out indicated therapeutic measures. (Rarely will this be possible in rural areas.)

It is not advisable in rural practice to induce labor early, or to carry out cesarean section in an attempt to minimize damage to the baby. Disadvantages of prematurity offset any advantage gained, especially when facilities for the premature are not ideal.

Efforts to prevent maternal antibody formation and to block action of the antibodies in the fetus have not been very successful. Some of the many agents tried are methionine, vitamin K, liver extract, progesterone, the hapten of Carter, cortisone and adrenocorticotrophic hormone (ACTH). The latter three can possibly do harm and should not be used in rural practice.

SIGNS OF ERYTHROBLASTOSIS IN THE INFANT

The pathogenesis of erythroblastosis has already been touched upon. In some manner erythrocytes of the fetus reach the maternal circulation. If the mother is Rh-negative, she may be stimulated to produce Rh antibodies which pass easily into the fetal circulation where they may cause hemolysis of fetal erythrocytes. The fate of the fetus, depending on the degree of hemolysis, varies from intra-uterine death to being born alive only to have hemolytic

crisis follow. The demand to produce erythroblasts may be so great that immature nucleated cells (erythroblasts) appear in the circulation of the fetus. The liver, spleen, and other organs may assume erythropoietic functions. This leads to the greatly enlarged liver and spleen often observed in babies with erythroblastosis.

Clinically, the symptoms may be of one of three forms of the disease:

1. *Hydrops fetalis* is the most severe form. The baby has severe generalized edema, dome-like abdomen and huge liver and spleen. There is no effective treatment. The baby is usually born a month prematurely and, if born alive, soon dies.

2. *Icterus neonatorum* is the form in which the infant, at birth or soon after, has severe hemolytic anemia and jaundice. There may or may not be hepatomegaly and splenomegaly. The mortality rate for babies with disease of this type (untreated) is more than 50 per cent. Exchange transfusion has reduced this figure to as low as 2 per cent.⁸

3. *The anemic type* is the mildest form. The problem is that of severe anemia which may develop rapidly or be slowly progressive over a period of weeks. The mortality rate if the baby is not treated is around 25 per cent. Perhaps all babies with this form of the disease could be saved by adequate care.

Laboratory determinations supporting a diagnosis of erythroblastosis may be divided into (1) those present in the prenatal period, such as maternal antibodies and x-ray films in which the fetus is observed in a Buddha-like position with a halo shadow about the scalp due to edema; and (2) postnatal data, including a positive result of the Coombs test, less than 16 grams of hemoglobin per 100 cc. of cord blood, a ratio of more than five immature nucleated erythrocytes per 100 leukocytes, and bilirubin content of over 3.0 mg. per 100 cc. of cord blood.

MANAGEMENT OF ERYTHROBLASTOTIC INFANTS

In the management of an erythroblastotic infant, at birth the cord should be clamped long and the cord blood collected for a Coombs test, for hemoglobin determination and for cell study. The baby should be placed in an incubator with oxygen flow on. Since many erythroblastotic infants appear normal at birth but within a few hours have severe anemia and jaundice necessitating immediate transfusion, it is important to determine the hemoglobin content of the blood every six to eight hours the first day and daily for a few days thereafter. Injections of vitamin K and liver extract may be of supportive value. Prophylactic penicillin may be desirable. Theoretical danger of antibody transfer in breast secretions is no longer thought to be a contraindication to breast feeding.

There may be some risk in a small transfusion, beyond that of inadequacy, since adult serum in transfusion may contain sufficient activating substance (conglutinin) to precipitate hemolytic crisis by activating the blocking antibodies coating the red cells of the baby.¹⁰ Exchange transfusion is more

efficacious. The dangers must be looked upon as a calculated risk justified by the circumstances.

Exchange transfusion appears to be lowering the incidence of kernicterus and neurological sequelae as much as it is reducing the mortality rate. Intelligence ratings of children who recover from erythroblastosis are only slightly lower than the average.⁴ It is so little lower that a physician has warranty for reassuring parents of infants recovering from the disease.

EXCHANGE TRANSFUSION

Factors to be considered in evaluating the status of an erythroblastotic baby are the hemoglobin value in the cord blood, the presence of jaundice, edema, respiratory difficulty, and neurologic abnormalities, the number of nucleated erythrocytes, and the degree of hepatosplenomegaly. There are no infallible rules, but in general exchange transfusion is probably indicated if any two of the following phenomena occur in a baby born to an Rh-negative mother: (1) Positive reaction to the Coombs test; (2) more than five to ten nucleated erythrocytes per 100 leukocytes; (3) hemoglobin content in cord blood less than 16 gm. per 100 cc.; (4) jaundice, or bilirubin content in cord blood over 3.0 mg. per 100 cc.

Infants who have erythroblastosis of the previously mentioned anemic type probably should have small transfusions (10 cc. of whole blood per pound of body weight). However, they should be given an exchange transfusion if respiratory distress develops concomitantly with a rapid decrease in hemoglobin and the number of erythrocytes in the blood.

Routine Rh-typing of obstetrical patients will have accumulated a list of potential Rh-negative donors. There are indications that a female donor is better for exchange purposes.¹ Group O Rh-negative blood which is compatible and shows no sensitization to the Rh antigen is acceptable. It is a probable choice in a rural community. Addition of commercial AB substance may enhance its safety. Fresh blood probably has some advantage over bank blood.

Procedure and Technique

Five hundred cubic centimeters of suitable donor blood is drawn. For exchange transfusion, catheterization of the umbilical vein of the baby is relatively easy during the first 12 to 24 hours. An 18 gauge polyethylene catheter (.067 inch outside diameter) is passed gently through the umbilical vein into the inferior vena cava until a free flow of blood is established. A blunt 18 gauge needle fits snugly in the open end of the catheter and is attached to two three-way stop cocks connected in series. Sterile tubing is connected to each valve, one leading to the transfusion set and the other to a waste receptacle. Then 20 to 25 cc. of blood is withdrawn from the baby and discarded. It is replaced with an equal amount of transfusion blood, and withdrawal and replacement then are alternated until 500 cc. has been replaced. This is said to accomplish replacement of 85 per cent of the baby's erythrocytes. To prevent hypocalcemia and tetany due to large amounts of citrate in the transfused blood, 1 cc. of a 10 per

cent solution of calcium gluconate is injected after each 100 cc. of blood is replaced.⁹ The apparatus should be flushed with saline solution to prevent clogging. Several syringes should be available. Some physicians advise the use of 0.2 cc. of heparin solution during the first half of the transfusion to prevent clogging.

The umbilical vein may thrombose early. If so, the saphenous vein technique is equally good² and is preferred by some investigators. A small incision is made one finger's breadth below the inguinal ligament, midway between the pubic tubercle and the anterior superior iliac spine. After the skin and subcutaneous fat are spread, the saphenous vein may be seen through a superficial layer of fascia. The vein is then isolated and ligated distally and opened gently with a needle or by small incision. The polyethylene catheter is introduced upward for a few inches until there is a free flow of blood. The catheter then is connected with the transfusion apparatus, as previously described, and the exchange is carried out. Replacement transfusion should be done slowly to avoid cardiac embarrassment. The venous pressure can be checked by placing a stainless steel millimeter rule vertically on the baby's xiphoid process and holding the plastic tubing against it. Normal pressures at the xiphoid process are not greater than about 6 cm. of blood. This refinement is not essential if three to four hours is given to doing the transfusion. There should be no hesitancy to discontinue the transfusion if, after infusion of a few hundred cubic centimeters of blood, the baby seems to be in distress.

The feasibility of such a procedure in a small community is illustrated in the following case reports.*

CASE REPORTS

CASE 1. A 34-year-old Rh-negative white pregnant female, married to an Rh-positive husband, had had four previous pregnancies with these results: Miscarriage at three months; term delivery of a stillborn; delivery of a hydropic fetus at eight months; intra-uterine death at six months with maternal blocking antibody titre of more than 1:500.

At eight and one-half months she was delivered of a living female infant with hepatosplenomegaly and, within a few hours, severe jaundice and respiratory distress. In desperation, 450 cc. of Rh-negative, Group O blood was given as a replacement transfusion through the umbilical vein. The baby improved rapidly and at ten months of age was apparently normal.

CASE 2.—A 24-year-old white female, Gravida II, gave a history of 76 hours in labor terminated by high forceps delivery of a living female infant that had severe "birth injuries" and lived a few months in a state of vegetation. The mother, critically ill with bilateral "milk leg," in an emergency transfusion was given a pint of Group O blood not typed for the Rh factor. (The patient had Group B blood.)

Upon the first examination in the second pregnancy (third month of gestation) the Rh-negative status was noted in routine Rh typing. Upon further study the presence of circulating agglutinins was noted. At the seventh month, agglutinating antibody titre was at 1:16 dilution and blocking antibody was up to 1:10. At term, cesarean section was done because the pelvis was relatively small and in light of the previous history. An apparently normal female infant was delivered. Positive reaction to a Coombs test of the cord blood was the only sign of erythroblastosis. However, jaundice began to develop within a few hours, and at ten hours the hemoglobin content of the blood was down to 10 gm. per 100 cc. and the number of erythrocytes had dropped from 4.5 to 3.5 million. An exchange transfusion was carried out. When attempts to get into the umbilical vein were not successful because of thrombosis, the saphenous vein technique was followed. There was progressive improvement after transfusion. Examined at five months of age, the baby seemed to be normal in all respects.

The first of the two case reports illustrates isoimmunization by pregnancy. In the second, sensitization undoubtedly was caused by a blood transfusion. Rh typing should be considered quite as important as blood grouping. No longer can infusions of whole blood without Rh typing be justified. This applies particularly to females before and during the age of childbearing.

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* From the author's practice at Merced, California.